

**IN VITRO CYTOTOXICITY STUDIES ON HYDRO-ALCOHOLIC AERIAL PARTS
EXTRACT OF *SESAMUM PROSTRATUM* RETZ**

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ABSTRACT:

One of the deadliest diseases threatening the human being is Cancer. Cancer is the second leading cause of death globally and among which Breast cancer is second leading cause of death in women. Breast cancer may be provoked due to several physical, chemical and environmental factors. Moreover, genetic alterations that are inherited via generation may be a reason for the occurrence of cancer. If breast cancer is not treated during initial stages, it may spread to nearby organs, a process called metastasis, through the blood or lymph system. The rising burden of cancer worldwide calls for an alternative treatment solution. Medicinal plants serves as an important tool for the treatment of various diseases and disorders. Herbal medicine provides a very feasible alternative to western medicine against cancer. This could be an alternative to other treatment methods, such as they cause minimal side effects when compared to chemo-drugs. In the present study, in vitro cytotoxic effect of hydro-alcoholic extract of *Sesamum prostratum* Retz. Aerial parts were evaluated against normal and cancer cell lines at five different concentrations such as 5 µg/ml, 25 µg/ml, 50 µg/ml, 75 µg/ml and 100 µg/ml. The percentage viability was found to be increasing with increasing concentration of test compound against the normal (L929) and cancer (MCF-7) cell lines. The extract was found to be cytotoxic against normal and cancer cell lines. The study indicates that, aerial parts can be better crude drug of choice against Human Breast cancer. The results revealed that, hydro-alcoholic extract *Sesamum prostratum* Retz. Have better IC₅₀ value 63 µg/ml against MCF-7 (Human Breast Cancer) cell line than the normal cell line IC₅₀ 30 µg/ml.

KEYWORDS: *Sesamum prostratum* Retz., MCF-7 cell line, L929, Cancer, MTT assay, Cytotoxicity.

INTRODUCTION:

Cancer is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body. Over 100 types of Cancers affect humans. According to data from the Global Cancer Statistics for the most frequent malignancies, breast and lung cancers were the most frequent cancers or worldwide,

accounting for 12.5% and 12.2% of all new cases diagnosed in 2020 [Sung H et al., 2021]. Breast cancer is the most commonly occurring cancer in women and the second most common cancer overall in terms of fatality after lung cancer [Bray F et al., 2018]. The rate of breast cancer patient's cases increasing every year, both in developed and developing countries [Torre L.A et al., 2015]. It is estimated that 26

million new cancer cases and 17 million cancer deaths are likely to occur per year globally by 2023 [Thun M.J et al., 2010]. In Asia alone, the incident from 2008 to 2030 is estimated to increase from 6.1 to 10.7 million deaths [Sankaranarayanan R et al., 2014]. Treating cancer has become a whole new area of research. There are conventional as well as very modern techniques applied against cancers. A variety of techniques i.e., chemotherapy, radiation therapy, or surgery are used for treating cancer [Karpuz M et al., 2018]. The advances of current treatments and detecting methods have contributed to the increased survival rate [Blumen H et al., 2016]. Unfortunately, these treatments cause short term and long term side effects for the patients. Moreover, studies have demonstrated that the burden of the cost related to cancer treatment is uncharacteristically rising. But as the problem persists, new approaches are needed for the control of diseases, especially, because of the failure of conventional chemotherapeutic approaches. Therefore, there is a need for new strategies for the prevention and cure of cancer to control the death rate because of this disease [Trogon J.G et al., 2017; Tariq Khan et al., 2020].

According to the World Health Organization (WHO), many countries, including developing countries, still use plants and natural source-related products for therapeutic purposes [Rayan et al., 2017]. About 60% of anticancer agents have been originated from the natural sources globally [Newman D.J and Cragg G.M, 2014]. Because herbal medicine has become a very safe, non-toxic and easily available source of cancer treating compounds. Herbs are believed to

neutralize the effects of diseases in a body because of various characteristic they possess [Cheng H, 1995].

Natural medicines, which come from plants, animals and minerals are one of nature's gifts to people and help fight many diseases [Tang C et al., 2020]. Plants have always been the foundation of traditional medicine and they have been helping people for thousands of years [Arpita Roy et al., 2017]. The pedaliaceae (pedalium family or sesame family) is a family of flowering plants with about 25 genus and 204 species (according to the WFO plant list), which grow in tropical and Southern Africa, South East Asia, and tropical Australia [Aguoru et al., 2016]. Most of the plants in this family are herbs that live for one year or more, and shrubs are rare. It was said that this family had medicinal properties, such as antimicrobial, antioxidant, anticancer, demulcent, emollient, and laxative etc...[Cathrine Chidewe et al., 2017]. *Sesamum* is a member of pedaliaceae family. This genus of plants is used to treat a wide range of illnesses.

Therefore, the main purpose of the study was to evaluate the anticancer effects of *Sesamum Prostratum* Retz. Aerial parts hydro-alcoholic extract on normal (L929) cell line and Breast Cancer cell line (MCF-7) by MTT assay.

MATERIALS AND METHODS:

Plant Material Collection:

Sesamum prostratum Retz. Plant were gathered in the Tamil Nadu region of Edappadi and authenticated by Dr.S.S.Hammed, Scientist "E" & Office in charge, Southern Regional Centre of the Botanical Survey of India, Coimbatore.

Preparation and Extract:

Sesamum prostratum stems, flowers, and leaves were gathered, cleaned and mechanically grinded. To prepare the crude extracts, maceration is used. Plant materials were coarsely powdered and soaked in hydro-alcoholic (ethanol:water (50:50)) and aqueous extraction solvents for seven days, agitated regularly and then filtered. After drying in hot air oven, the extracts are placed in approximately labeled vials and stored in the refrigerator. The hydro-alcoholic and aqueous extract yielded 17.865 (w/w) and 29.21% (w/w), respectively, when the dry extract was weighed.

Phytochemical Screening:

The phytochemical composition of *Sesamum Prostratum* aerial part hydro-alcoholic extract was examined in order to evaluate their qualitative chemical components. The components were screened according to Trease and Evans [Trease G.E and Evans W.C, 1989] and Sofowora [Sofowora A, 1993]'s description of the approach process, and reagents employed.

IN-VITRO ASSAY FOR CYTOTOXICITY ACTIVITY (MTT ASSAY)

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0×10^5 cells/ml using DMEM medium with 10% FBS. Each well of the 96 well microtitre plate was added with 0.1ml of the diluted cell suspension (approximately 10,000 cells). After 24 hr, a partial monolayer was formed. The supernatant was flicked off,

the monolayer was washed with medium and 100 μ l of different test concentrations of *Sesamum prostratum* Retz. Successive extract of hydro-alcoholic were added on to the partial monolayer in microtitre plates. The plates were then incubated at 37°C for 72 hr in 5% carbon dioxide atmosphere. The microscopic examination was carried out periodically and observations were noted every 24hr interval. After 72 hr, the test drug solutions in the wells were discarded and 50 μ l of MTT in PBS was added to each well. The contents of the plates were gently shaken and incubated for 3hr at 37°C in 5% carbon dioxide atmosphere. The supernatant was removed and to plates 100 μ l of propanol was added and again the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540nm. The percentage growth inhibition was calculated using the following formula and concentration of *Sesamum prostratum* Retz. Extract needed to inhibit cell growth by 50% (CTC50) values is generated from the dose-response curves for both cell line [Francis D and Rita L, 1986; Philip Skehan et al., 1990; Unnikrishnan Ramadasan Kuttan M.C, 1988].

Percentage growth inhibition = $100 - \frac{(\text{Mean OD of individual group} \times 100)}{\text{Mean OD of Control group}}$

RESULTS AND DISCUSSION:

Phytochemical Screening:

Flavonoids, carbohydrates, steroids, and proteins were found in hydro-alcoholic plant extracts after preliminary phytochemical analysis (Table 1).

Table No 1: Phytochemical constituents of investigated plant extracts

S.NO	Phytoconstituents	Hydroalcoholic extract
01	Alkaloids	-
02	Glycosides	+
03	Flavonoids	+
04	Tannins	+
05	Steroids	+
06	Carbohydrates	+
07	Proteins and Amino acids	+
08	Fats and fixed oils	-
09	Vitamin C	-

‘+’; Present, ‘-’; Absent

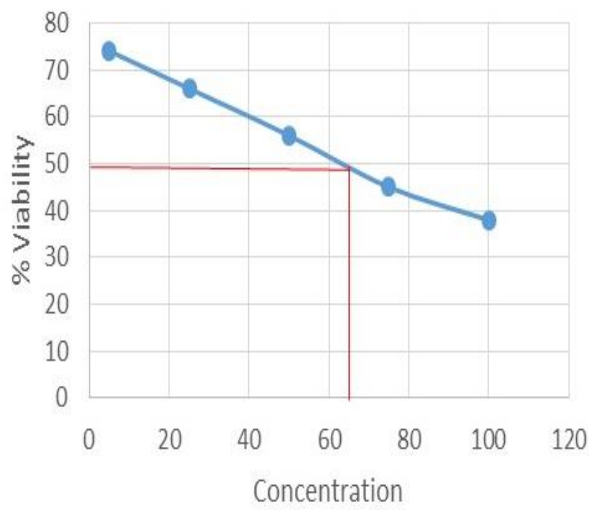
IN-VITRO CYTOTOXIC ACTIVITY

Table No 2: Cytotoxicity effect of hydro-alcoholic extract of *Sesamum prostratum* Retz. Aerial parts

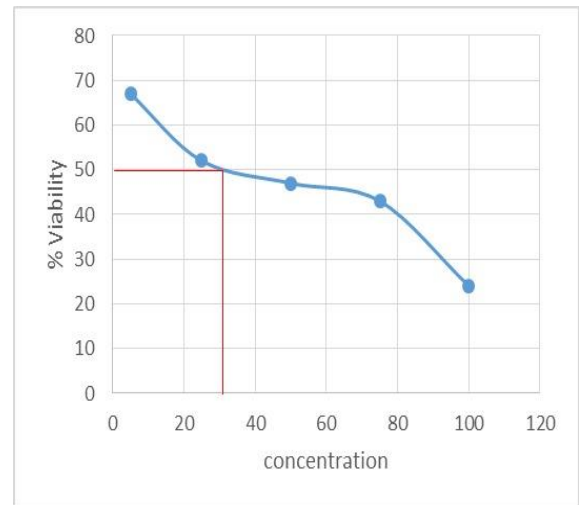
S. No	Cell lines	Test concentration (µg/ml)	Cytotoxicity (%)	% viability	IC ₅₀ (µg/ml)	Reactivity
1.	L929	5	26	74	63	Mild
2.		25	34	66		Mild
3.		50	44	56		Mild
4.		75	55	45		Moderate
5.		100	62	38		Moderate
6.	MCF-7	5	33	67	30	Mild
7.		25	48	52		Mild
8.		50	53	47		Moderate
9.		75	57	43		Moderate
10.		100	76	24		Severe

In the present study, in vitro cytotoxic effect of hydro-alcoholic extract of *Sesamum prostratum* Retz. Aerial parts were evaluated against normal and cancer cell lines at five different concentrations. The percentage viability was found to be increasing with increasing concentration of test compound against the normal and

cancer cell lines. The study indicates that, aerial parts can be better crude drug of choice against Human Breast cancer. The results revealed that, hydro-alcoholic extract *Sesamum Prostratum* Retz. Have better IC₅₀ value against MCF-7 (Human Breast Cancer) cell line that the normal cell line by exhibiting an IC₅₀ of 30 µg/ml.



(a)



(b)

Graph No 1: (a) Graphical Representation of Hydro-alcoholic extract on L929

(b) Graphical Representaion of Hydro-alcoholic extract on MCF-7

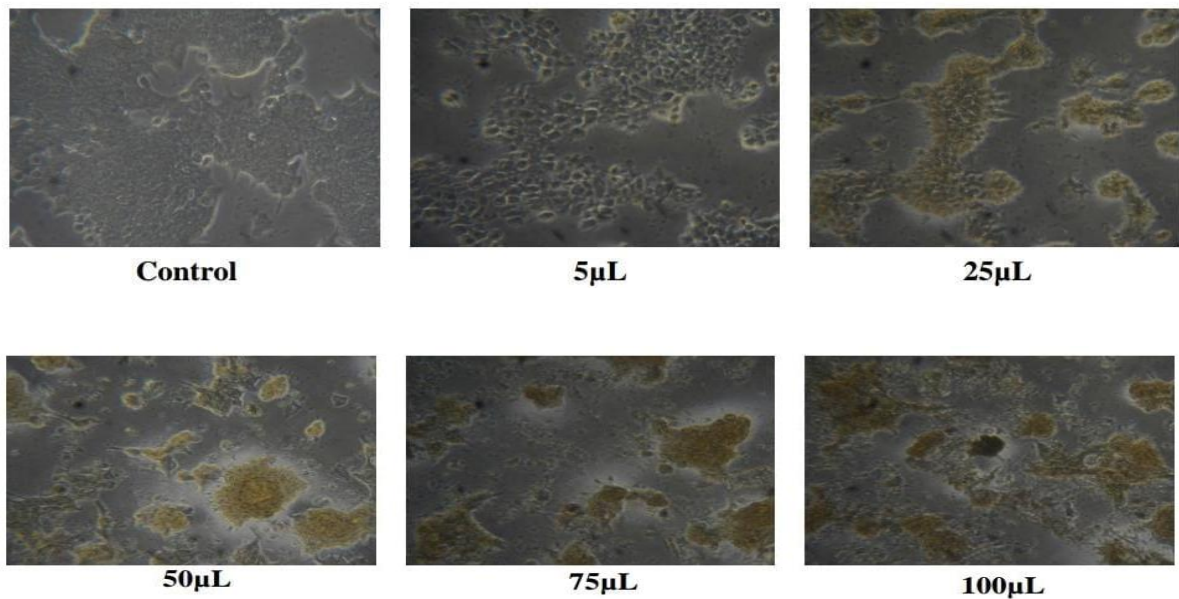


Fig No 1: Cytotoxicity effect of *Sesamum Prostratum* Retz. Hydro-alcoholic extract against MCF-7 (Human Breast Cancer cell line)

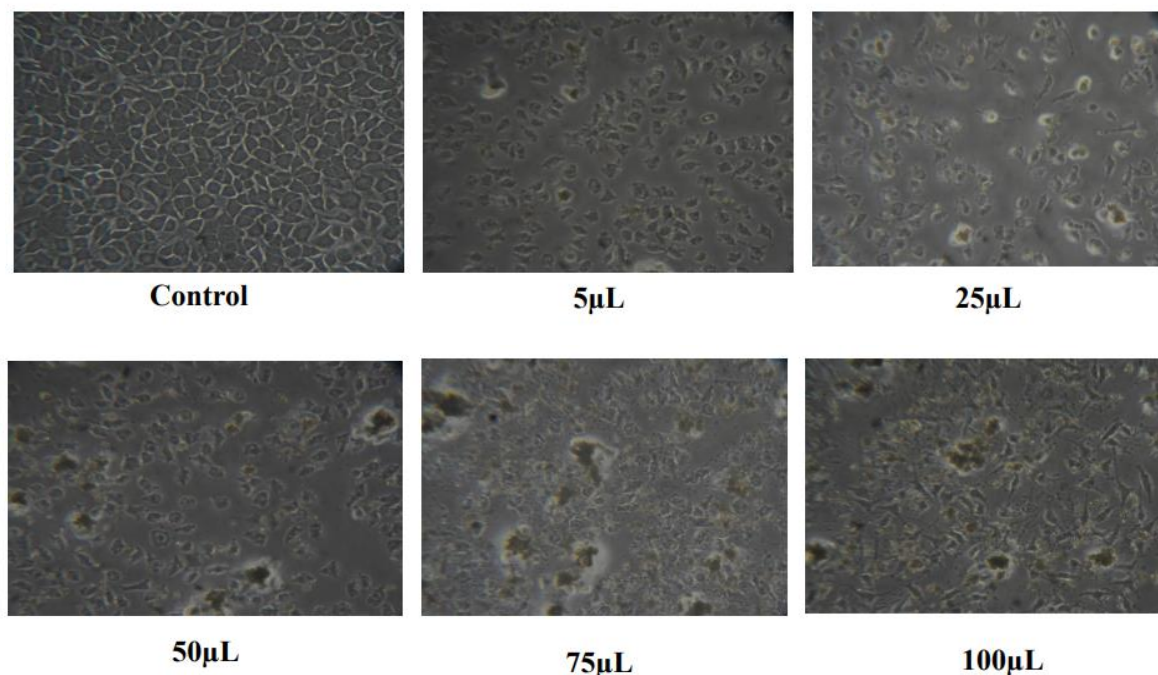


Fig No 2: Cytotoxicity effect of *Sesamum Prostratum* Retz. Hydro-alcoholic extract against L929 (Normal cell line)

CONCLUSIONS:

Preliminary phytochemical evaluation of hydro-alcoholic extract of *Sesamum prostratum* Retz. Aerial parts revealed the presence of flavonoids, glycosides, tannins, steroids, carbohydrates and proteins and amino acids. Further *Sesamum prostratum* Retz. Aerial parts were evaluated for its cytotoxic potential by MTT assay against normal (L929) and cancer (MCF-7) cell lines. The hydro-alcoholic extract is found to be cytotoxic against normal and cancer cell lines. The hydro-alcoholic extract *Sesamum Prostratum* Retz. Have better IC_{50} OF $30\mu\text{g/ml}$. the results shows, the aerial parts can be better drug of choice against human breast cancer cells. Further, isolation and characterization of active phytoconstituents from the aerial parts may leads to a novel drug of choice against human breast cancer. *Sesamum prostratum* Retz. Is potentially beneficial for cancer prevention.

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